Mutation Analysis Significantly Improves the Diagnostic Utility and Patient Management of Cytologically Indeterminate Thyroid Nodules

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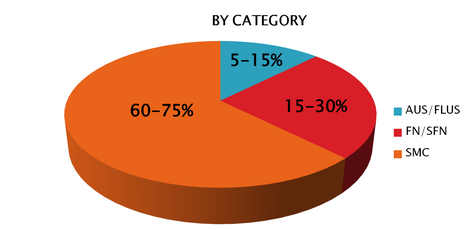
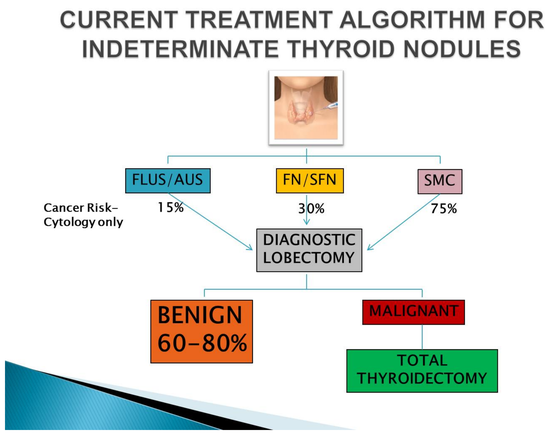
INTRODUCTION

- Thyroid nodules are common in adults, but only a small fraction of them are malignant.
- Fine-needle aspiration (FNA) cytology provides a diagnosis of benign versus malignant disease in majority of cases.
- However 25% of nodules are cytologically indeterminate (figure 1), thereby needing repeat FNA or diagnostic lobectomy for definitive diagnosis.
- Aim of this study was to evaluate diagnostic and clinical utility of molecular testing using the newly launched Entrogen thyroid cancer mutation panel

Figure 1: Risk of malignancy based on cytology diagnosis



Indeterminate thyroid nodules by Cytology include:
•AUS / FLUS: Atypia of undetermined significance / Follicular lesion of undetermined significance
•FN / SFN: Follicular neoplasm / Suspicious for follicular neoplasm
•SMC: Suspicious for malignancy



MATERIALS AND METHODS

- 21 cytologically indeterminate samples were used.
- 16 samples were collected in Cytolyt™ preservative, 2 in PreservCyt™ and 3 samples tested had both Cytolyt™ and FFPE cell block.
- In addition, 2 FFPE blocks of positive controls (RET/PTC1 and PAX8/PPARγ) along with 12 commercially available FFPE standards were also tested.
- ***16 most common mutations in BRAF, KRAS, NRAS and HRAS and 3 rearrangements which include RET/PTC1, RET/PTC3 and PAX8/PPARγ are detected by this assay (figure 2)***
- Cytolyt™ and PreservCyt™ samples were spun to discard supernatant and total nucleic acid was extracted from the pellet using Promega total viral nucleic acid kit.
- DNA and RNA were individually extracted from FFPE blocks.
- Purity and concentrations were measured using QuantiFluor®.
- Assay input for DNA mutations was between 5-10 ng per reaction and RNA was 50 ng per reaction.
- Assay sensitivity was evaluated by serial dilutions of the commercial standards using wild-type DNA.
- Results were compared with cytology diagnoses.

Figure 2: Alterations detected by Thyroid mutation panel

Gene	aa change	nt change	Cosmic ID	Detected by Primer No.
BRAF	V600E	c.1799T>A	476	1
KRAS	G12A	c.35G>C	522	2
	G12D	c.35G>A	521	
	G12R	c.34G>C	518	
	G12V	c.35G>T	520	
	G13D	c.38G>A	532	
	G12C	c.34G>T	516	3
	G12S	c.34G>A	517	
NRAS	Q61H (CAC)	c.183A>C	585	4
	Q61H (CAT)	c.183A>T	586	
	Q61L	c.182A>T	583	
	Q61K	c.181C>A	580	
	Q61R	c.182A>G	584	
HRAS	G12V	c.35G>T	483	5
	G13R	c.37G>C	486	
	Q61R	c.182A>G	499	

RESULTS

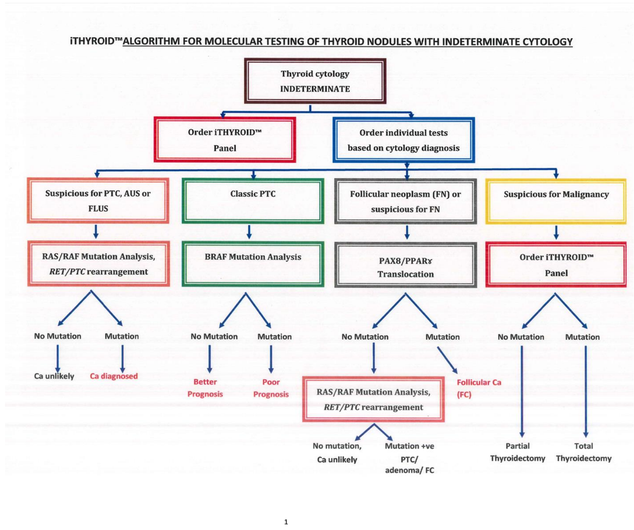
- 3 samples were positive for BRAF V600E mutation, 2 of which were suspicious for papillary carcinoma on cytology. (figure 3)
- One sample that was diagnosed as atypia of undetermined significance was positive for NRAS codon 61 mutation where as one suspicious for follicular neoplasm was positive for HRAS mutation.
- The samples that were negative for mutations and translocations were cytologically either atypia of undetermined significance or follicular lesion of undetermined significance.
- The assay sensitivity was 1% for all mutations except G12R/G12S/G13D (5%) and up to 0.78% for RET/PTC1 and PAX8/PPARγ.
- No cross-reactivity was seen with any of the probes.
- Total thyroidectomy performed for patients with positive mutation showed papillary thyroid carcinoma (PTC) (n=3), follicular variant of PTC (n=1) and follicular carcinoma (n=1).

Figure 3: Summary of results using Thyroid mutation panel

Sample ID	Collection Medium	Cytology Diagnosis	Molecular Alterations	Final Diagnosis on Surgical specimen
1	Cytolyt™	AUS	None Detected	N/A
2	Cytolyt™	AUS	None Detected	N/A
3	Cytolyt™	FLUS	None Detected	N/A
4	Cytolyt™	SMC	*BRAF* V600E	Papillary carcinoma, vascular invasion
5	Cytolyt™	AUS	None Detected	N/A
6	Cytolyt™	SMC	None Detected	N/A
7	Cytolyt™	FLUS	None Detected	N/A
8	PreservCyt™	AUS	None Detected	N/A
9	Cytolyt™	AUS	*BRAF* V600E	Papillary carcinoma, 1 node positive
10	Cytolyt™	SFN	None Detected	N/A
11	Cytolyt™	AUS	NRAS codon 61	Follicular Adenoma, intact capsule
12	PreservCyt™	AUS	None Detected	N/A
13	Cytolyt™	AUS	None Detected	N/A
14	Cytolyt™	SMC	*BRAF* V600E	Papillary carcinoma, capsular invasion
15	Cytolyt™ & FFPE	AUS	None Detected	N/A
16	Cytolyt™	FLUS	None Detected	N/A
17	Cytolyt™	AUS	None Detected	N/A
18	Cytolyt™ & FFPE	SFN	HRAS	Patient did not opt for lobectomy
19	Cytolyt™	AUS	None Detected	N/A
20	Cytolyt™ & FFPE	AUS	None Detected	N/A
21	Cytolyt™	AUS	None Detected	N/A

RESULTS

Figure 4: Testing algorithm based on cytology diagnosis implemented by CPA lab



CONCLUSIONS

- ❖ Thyroid cancer mutation panel is highly sensitive for detection of common genetic abnormalities seen in up to 50% of thyroid carcinomas.
- ❖ This test can be performed on routine cytology as well as FFPE tissue and does not require special collection medium or preservative.
- ❖ It improves the diagnostic yield of cytology and can therefore help in effective clinical management.
- ❖ Better preoperative stratification of patients with thyroid nodule malignancy risk
- ❖ Improved ability to guide initial definitive (partial/total) thyroidectomy